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An Investigation of Factors Affecting the Incidence of Lenticel Infection of Apples by Penicillium Expansum

by

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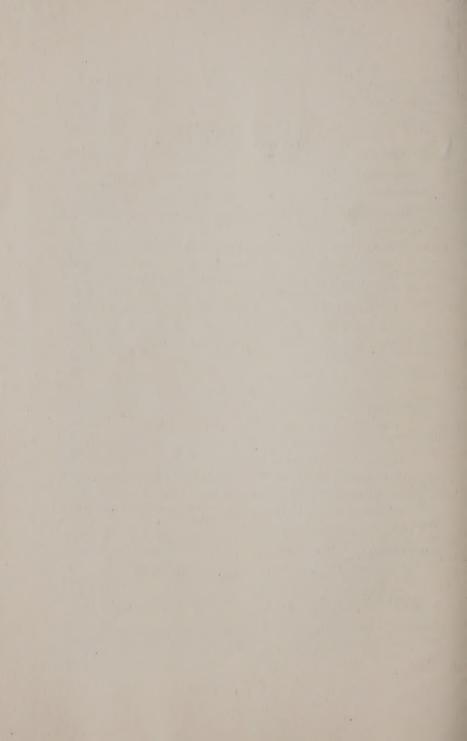[2] In cooperation with the United States Department of Agriculture.
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Kenneth F. Baker[2] and F. D. Heald[3]

INTRODUCTION

Many investigators in the various apple-producing areas of the world have reported *Penicillium expansum* Link as the most important cause of decay in stored apples. Cunningham (18) stated that soft rots (largely caused by *P. expansum*) take an annual toll of 15 per cent of the crop in New Zealand. In the United States this fungus is believed to cause 75 to 95 per cent of the decay of apples in storage (12, 16, 22, 37, 52). Some idea of the losses sustained in Washington may be gained from the fact that 1.5 per cent of the fruit in cars inspected at eastern terminals in the five-year period, 1925-1929, was rotted by blue mold (4, 36, 65). Extending this conservative figure to the United States as a whole, in which the annual commercial[4] apple crop was valued at $111,283,380[5] during the period, the annual loss for the apples *actually decayed* would exceed $1,600,000. The actual losses sustained are much greater, for they include the cost of harvesting, packing, shipping, and frequently of re-packing, as well as lowering of grade and of the general price level, slowing up of demand, damage to reputation, claims for damage, and dumping of fruit. The losses in fruit not of the commercial crop are even greater. Fisher (22) stated that "It is impossible to secure accurate estimates of losses in local markets and in home storage, but there is little question but that this amounts to over 10 per cent of the crop thus handled."

The lenticels of apples commonly serve as infection courts for *P. expansum*. Since the average amount of blue-mold decay in car-lot shipments of Washington apples has remained fairly uniform since 1922, in spite of the introduction of better methods of handling in that time, it is thought that lenticel infection, a type of entrance not affected by these improvements, determined the minimum amount of decay (4). The importance of this means of entry in the Washington apple crop (4, 37) is such that the factors predisposing to its occurrence have been investigated with the ultimate view of possible control.

The experimental work here reported was carried on in the Yakima and Wenatchee Valleys, and in the Plant Pathology laboratories in Pullman, from September 1930 to March 1934.

LITERATURE REVIEW

The first report of the occurrence of *Penicillium expansum* on decayed pome fruit is to be found in the "Observationes" of Link (44) in 1809, in which the genus was erected. However, Davaine (19) in 1866 was the first to prove definitely that the organism was the primary cause of the decay, and not secondary to chemical changes.

The market apples of 50 years ago probably had more injuries of the epidermis and cuticle from mechanical sources, stigmonose, and the attacks of fungi in the orchard, than the fruit of today. As infections at injuries were the most obvious and abundant, wounds came to be regarded as the only means of entrance. This interpretation has been the prevalent one found in the literature on blue-mold decay.

Zschokke (72, 73) in Switzerland was the first worker to recognize infections by blue mold through lenticels. Kidd and Beaumont (41, 42) studied the problem of lenticel invasion by decay fungi in England, and concluded that penetration by *P. expansum* occurred "only rarely through a lenticel." After an investigation of this phenomenon they concluded that "The important factors controlling fungal penetration through the lenticel in storage would appear to be those which affect the germination and growth of the fungal mycelium outside the host, namely, moisture, nutrition, and temperature." They thought that the "local physiological breakdown of the apple tissue in the neighborhood of the lenticels may supply sufficient moisture and nutrient for the fungus to germinate."

Brooks (13) recognized the occurrence of infection at lenticels by *P. expansum*. Balachonov (7) mentioned the infection of apples through areas of uninjured cuticle from which the natural waxy coating was rubbed.

Heald and Ruehle (37) reported the occurrence of lenticel infections by *P. expansum* in the commercially stored apple crop for the first time in the United States. The first survey of commercial storages in this country to determine the importance of lenticel infection was made by

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the writers (4). Apples of Jonathan, Delicious, Winesap, Rome Beauty, Arkansas Black, Spitzenburg, King David, and Yellow Newtown varieties produced near Wenatchee, Prosser, and Yakima, showed blue-mold decay starting at the lenticels in up to 33.3 per cent of the fruit. Apples of all sizes in both washed and unwashed lots showed this mode of entrance of the fungus. It was thought that the realization of the importance of this type of infection would suggest new methods of reduction of decay.

Sardiña (60) reported blue-mold infections at lenticels as common on apples in Madrid markets and storages. He obtained such infections experimentally by rubbing the fruit with spores.

The ability of blue mold to infect sound fruit through lenticels by growth from adjacent rotten apples has been frequently reported (2, 12, 22, 53). The explanation of Kidd and Beaumont (42) for lenticel invasion by fungi should be considered in this connection. Fungi were thought to be able to develop at a lenticel to a degree dependent on the "vigor of development" of the organism, which was in turn affected by the senescence of that area of the fruit. The readiness of direct spread from a decayed fruit to an uninjured normal one might then be due to its vigorous growth under those conditions.

STRUCTURE OF LENTICELS

In this discussion the term *lenticel* is used advisedly in reference to the "fruit dots" of apples, for most of them are lenticel-like rather than of the structural type found on stems. The histology of these structures has been investigated by various workers (3, 10, 24, 26, 42, 48, 66, 67, 72, 73), and the nature of the cuticle and epidermis has been studied to some extent (49, 50, 54, 55, 67). A brief summation of their results is given as a basis for the discussions to follow.

The epidermis of young apples has many unicellular hairs and stomata. The former are lost by breaking at the level of the epidermis or by loosening and dropping out of their "pockets" while the fruit is but a few weeks old. The cells under the stomata are modified soon after blossom fall; most of the lenticels arise from these structures. If the stomata are not split by the enlargement of the fruit they are to be seen persisting in the lenticel of the mature fruit. The epidermis consists of a single layer of cells with thickened external walls largely of cutin; these cells increase more tangentially than radially by both enlargement and division, but are unable to maintain their relation with the underlying tissue. In most cases a cutin deposit between the radial walls of epidermis cells will increase surface area without the formation of cork. A tangential tension of the epidermal structures results from the growth of the underlying tissue, the weak points at the stomata and hair pits naturally rupturing first. The rupture of the stomata may be prevented by the development of supporting cells around the guard cells, or a tear

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may occur between or at right angles to them before or after the formation of underlying cutinized cells.

The rupture of the stomata, hair pits, or epidermis initiates the development of structures of highly variable nature. These range from a separation of the cuticle and epidermal cells, exposing the underlying modified tissue, through the "Lentizellenähnlichen Korktüpfel" or structures having irregularly arranged cutinized[1] cells beneath the opening, to typical lenticels having definite layers of cutinized cells. The latter type is somewhat rare, but occurs on russet varieties (e.g. French and Portugese Lederreinette). The amount of cork present varies with the time of yea. and the variety. The regular or irregular layers of cutinized cells may crack, exposing the tissue beneath; this is most common in lenticels having several layers of cutinized cells. The openings are 60 to 300 microns wide and of very irregular shape. Lenticels are most abundant per unit area at the calyx end, on account of less expansion at that point than at the stem end. Their efficiency in gas exchange cannot be determined by macroscopical examination.

Submersion of apples in an aqueous solution of methylene blue for two or three days has ben found by Clements[2] to reveal three general types of lenticels on apples: (1) the open type, indicated by the penetration and diffusion of the dye in a "halo" around the lenticel; (2) the partly-open type, indicated by the absorption of the dye by the lenticel basin; (3) the closed type, which absorbs no dye. Histological study has shown that the open type includes structures in which the unmodified cells beneath are exposed either between cutinized cells or throughout the cavity. The partly open type includes structures with definite external openings and a completely suberized or cutinized basin which absorbs the dye to a limited extent. The closed type is represented by those without external openings, or with such openings and a cutinized basin which does not absorb the dye. These facts give presumptive evidence that the open type would be the most readily infected by fungi. Tetley (67) found many fungous hyphae in lenticels in which cracks extended through the cutinized layers of cells, but reported only a few hyphae in more shallow uncracked lenticels.

Devaux (20) concluded that lenticels of stems were altered by the antithetic processes of cicatrization and hypertrophy, which respectively sealed and ruptured the lenticular layers in such a way as partially to regulate the internal hydrostatic pressure and the rate of water loss. As the apple is an inferior fruit the conditions reported for stems may also apply to it.

Reed and Crabill (57) in the northern Shenandoah Valley, and Heald (35) in Washington, have reported a splitting of the skin, starting at

[1] Tetley (67) reported a tannin-like deposit in the lenticel basins.
[2] Unpublished investigations of Dr. H. F. Clements in the Department of Botany.

lenticels, resulting from a sudden abundant supply of water following a period of dryness. A cork layer subsequently developed under these cracks, but *Alternaria mali* Roberts frequently penetrated before its formation. It would seem that less severe cracking of lenticels could be caused in the same way, giving an inconspicuous infection court to various fungi.

MATERIALS, METHODS, AND RESULTS

Grower-lot Relations

The opinion is held by members of the apple industry that fruit grown on certain ranches (i.e. grower lots) has a tendency to show an abundance of blue-mold decay every year. In the examination of fruit in commercial storage in 1932 (4) and in random samples shipped to Pullman[1] in 1932 (Table 1) this belief was found to be justified in some cases. A few representative examples are cited below:

Lot 1. The Rome Beauty apples from a ranch on Englewood Heights were reported by a Yakima storage house to have had an abundance of decay in the 1929 and 1930 crops. An examination of random samples of the 1931 Fancy Rome Beauty crop showed 26.7 per cent blue-mold decay, 16.3 per cent being of lenticel origin.[2]

Lot 2. The fruit of a ranch near Wenatchee was reported by the company that had stored the fruit for several seasons as usually having a large amount of decay; the 1930 crop had to be repacked. Examination of random samples of the 1931 crop showed 5.0 per cent blue-mold rot (1.7 per cent infection at lenticels) in the Fancy Winesaps and 1.5 per cent blue-mold rot (0.1 per cent lenticel infections) in the Extra Fancy Arkansas Black apples.

Lot 3. A warehouse in Selah found severe decay during storage in the 1930 crop of Winesap and Rome Beauty apples from a local orchard, and did not handle the crop the following year for that reason. A Yakima fruit company reported blue-mold decay in amounts up to 40 per cent in the 1931 crop; random samples of the lot showed 27.2 per cent blue-mold rot, 16.9 per cent having started at lenticels. However, Winesaps (Table 12) of the 1932 crop showed but 1.1 per cent blue-mold rot and no lenticel infections; unwashed Jonathans showed 4.7 per cent blue-mold rot and 2.3 per cent lenticel infections.

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[1] Unless otherwise specified, apples used in Pullman were held at -0.5 to 1° C.

[2] In this paper, lesions which centered on lenticels and had no other visible place of entrance are tabulated as **certain** lenticel infections; those which did not start at an injury or necrotic area, or at the stem or calyx, but which were too large, wrinkled, or overgrown with the fungus for positive identification, are listed as **probable** lenticel infections (L. I.). As a large percentage of the latter class are undoubtedly lenticel infections they should be considered to some extent in evaluating the results presented.

Lot 4. The fruit of a ranch near Tieton was reported by a Naches storage house as showing considerable blue-mold rot every season while in their plant. Extra Fancy Delicious apples of the 1931 crop were found to have 32.9 per cent blue-mold rot (23.8 per cent lenticel infections). Jonathans of the 1932 crop showed 5.1 per cent blue-mold rot (3.6 per cent

Table 1. The Percentage of Apples Showing Blue-Mold Decay in 1932 in Commercially Packed Lots from Ranches Which Had Considerable Decay in Previous Years

Grower lot and location	Variety	Inoculum	Number apples	Per cent blue-mold decay		
				Total	Lenticel infections	
					Certain	Probable
4; Tieton[1]	Jonathan	Dipped in spore suspension	549	15.7	11.3	1.1
		Not dipped in spore suspension	276	5.1	3.6	0.7
7; Wiley Heights[2]	Delicious	Dipped in spore suspension	552	2.4	0.4	0.5
		Not dipped in spore suspension	269	0.4	0	0
8; Gleed[3]	Winesap	Dipped in spore suspension	474	2.5	0.6	0.6
		Not dipped in spore suspension	238	0.4	0.4	0
5; Selah	Winesap[4]	Dipped in spore suspension	501	6.8	2.2	1.1
		Not dipped in spore suspension	263	7.6	1.5	1.1
	Delicious[5]	Not dipped in spore suspension	726	5.9	2.2	2.2

[1] Fruit (Extra Fancy) washed in unheated 1½ per cent HCl about October 23; held 4 days in cold storage before dipped in spore suspension. Data taken December 21, 1932 and January 3, 1933.

[2] Fruit (Extra Fancy) washed in soda ash (50-60 lbs. per 100 gals.) at 32° C. on October 8; held 5 days in common storage before dipped in suspension of spores. Data taken January 5, 1933.

[3] Fruit (Extra Fancy) washed in Brogdite (1 lb. per gal.) at 48° C. about October 31, when dipped in spore suspension. Data taken February 24, and May 5, 1933.

[4] Fruit (Extra Fancy) washed in 1½ per cent HCl at 43° C. and dipped in spore suspension on October 31. Data given is total decay found in examinations on February 24 and May 4-5, 1933.

[5] Fruit (Extra Fancy) taken from a commercial cold storage in Yakima and shipped to Pullman. Method and time of washing unknown. Data taken December 22, 1932, and January 5, February 23, and May 4, 1933.

lenticel infections); Jonathans handled. in the same way, except dipped in a spore suspension to insure an abundance of inoculum, showed 15.7 per cent blue-mold decay (11.3 per cent lenticel infections).[1]

Lot 5. Fruit of the 1931 crop of another ranch near Selah showed amounts of blue-mold rot up to 20 per cent in Extra Fancy Winesap apples in a Gleed storage. Winesap apples of the 1932 crop showed 7.6 per cent blue-mold rot (1.5 per cent lenticel infections); Winesaps handled in the same way, except dipped in a spore suspension to insure an abundance of inoculum, showed 6.8 per cent blue-mold decay (2.2 per cent lenticel infection).[1]

Lot 6. The fruit of a ranch near Ahtanum showed an abundance of blue-mold rot in the crops of 1929, 1930, and 1931 while in a local fruit storage. The Winesap, Rome Beauty, and Delicious varieties showed much decay in the 1931 crop, but the Jonathans had only slight decay. Fruit from this orchard could not be obtained for examination, but reports of the Horticultural Inspector and the manager of the packing plant leave little doubt but that much of the infection started at lenticels.

Lot 7. The 1931 crop of a ranch on Wiley Heights (Yakima) showed a large amount of decay; one lot of Extra Fancy Winesaps reported to have up to 42 per cent blue-mold rot, showed 26 per cent blue-mold decay (20 per cent lenticel infections) in random samples; another lot showed 19.2 per cent lenticel infections by *Sporotrichum malorum* Kidd and Beaumont. Delicious apples of the 1932 crop showed 0.4 per cent blue-mold rot (no lenticel infections); Delicious handled in the same way, except dipped in a spore suspension to insure an abundance of inoculum, showed 2.4 per cent decay (0.4 per cent lenticel infections).[1]

Lot 8. Fruit from a ranch near Gleed showed an abundance of blue-mold infections at lenticels in the 1931 crop, according to Horticultural Inspectors and the owner. Fruit from this orchard generally had very little decay in other seasons. Winesaps of the 1932 crop showed 0.4 per cent blue-mold decay, all starting at lenticels; Winesaps handled in the same way, except dipped in a spore suspension to insure an abundance of inoculum, showed 2.5 per cent blue-mold rot (0.6 per cent infections at lenticels).[1]

Lot 9. Two ranches near Wenatchee have a reputation with a local produce company for producing fruit relatively free from decay. However, examination of C grade Delicious of the 1931 crop of one of these ranches showed 10.2 per cent blue-mold decay (2.2 per cent lenticel infections) and the other 11.1 per cent blue-mold decay (4.5 per cent lenticel infections).

[1] Table 1.

Several valuable leads were derived from this work. There seems to be a definite tendency of fruit from certain orchards (lots 1, 2, 3, 4, 5, and 6) to show a large amount of blue-mold decay every year, but in other cases (lots 3, in part, 7, 8, and 9) apples of a given grower lot may be severely decayed only in occasional years. Many grower lots designated by storage operators or Horticultural Inspectors as usually severely attacked by blue-mold were found to have little or no decay. In these cases the grower is frequently penalized in years of a crop with little decay (by forced early marketing of fruit or by reluctance of packers to handle the fruit at all) because of the uncertainty involved in handling, making the loss nearly as great as though the trouble occurred every year.

There is a distinct tendency over a period of years for fruit from certain ranches (lots 3 and 5) to show decay originating at lenticels, regardless of the packing house handling the fruit; this would suggest that conditions previous to arrival at the packing plant were responsible. In other instances the packing plant seems unquestionably to be at fault.

In some cases (lots 4, 7, and 8) an abundance of inoculum increased the number of lenticel infections, while in others (lot 5) the increase of the spore load did not increase the amount of decay. This suggests that two factors determine the number of blue-mold lenticel infections: (1) the condition of the lenticels; (2) the amount of inoculum. In cases where the amount of inoculum increases the number of lenticel infections the fruit has a considerable number of susceptible lenticels, and in cases where this does not increase infections the fruit has few or no susceptible lenticels.

These findings (particularly in lots 1, 3, 4, and 7) further substantiate the conclusion (4) that "Lenticel infection may be . . . the principal factor involved in lots of fruit showing high percentages of decay at eastern terminals."

Personal examinations of eight of the ranches found rather consistently to produce fruit high in blue-mold decay, and discussion with the managers of them, failed to show any factor or factors of culture, handling, spraying, etc., common to even a few of them, but did demonstrate the revealing fact that some growers (e.g. lot 1) were not aware that their fruit usually had an abundance of blue-mold decay. These facts supplied presumptive evidence to the hypothesis that a multiplicity of factors was involved in determining the susceptibility of lenticels to infections by *P. expansum.*

Factors Affecting the Incidence of Lenticel Infections

At the beginning of these studies it was thought that there might be one or two principal environmental factors affecting the susceptibility of the lenticels to infection and that control could be effected by modification of these. While it is realized that the evidence presented on the effect

of some of the factors is insufficient to establish their relative importance in predisposition to infection, the factors shown to influence this condition are to be considered as potentially important in any given lot. It is indicated that susceptibility is affected by a multiplicity of factors of the environment and of the fruit.

In 1931 a series of investigations was undertaken in the Experiment Station orchard at Wenatchee to determine the relation of the following five factors to invasion of lenticels by *P. expansum*:

1. Variety: Jonathan; Delicious; Winesap.

2. Time of picking: Early, prime, late, and extra-late maturity.

3. Inoculum: Dipped in spore suspension; not dipped in spore suspension.

4. Moisture conditions following picking: Held moist; held dry.

5. Time held in orchard following picking: Not held; held six days; held 12 days.

The 14 variable conditions of this series were arranged in all possible combinations (except in the extra-late picking), 111 packed boxes (13,881 apples) being used. In the tabulations of the 1931 crop all fruit (except that of the extra-late picking) of each variety dipped in a spore suspension and subjected to each treatment is considered in the computations. The average percentage of decay is determined by the action on one variety of one factor influenced by two other variable factors. This method of analysis gave a better conception of the relative importance of a given conditioning factor under field conditions than a tabulation of each variable factor with all others constant. None of the fruit used in this 1931 series was washed.

The Jonathan apples were obtained from five adjacent trees in a block of the orchard which was given the usual amount of irrigation and fertilizer and was in cover crop. The trees were given a calyx and five cover sprays with arsenate of lead plus Fluxit. The early-, prime-, and late-maturity, and extra-late pickings were made September 4, 15, 30, and October 5, 1931, respectively, and the pressure and color tests shown in Table 2 were made. Each of the lots of fruit picked was divided at once into two parts, one half being dipped in water and the other into a suspension[1] of blue-mold spores in water. Each of these portions was again divided into two parts; one was held dry in the picking boxes under a canvas cover in the orchard, while the other was held moist in picking boxes by being sprinkled every two or three days with water, and was also kept under a canvas cover. The humidity in the latter case

[1] Packets of spores of **P. expansum** in sugar placed in uniform amounts of water. Tests by dipping 10 apples, each having 3 punctures, in the suspension showed 100 per cent blue-mold decay in all cases. The cultures used in the studies presented in this paper were all transfers from the strain used in previous work (5).

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Table 2. Maturity of Fruit Used in Obtaining Data for Tables 3, 4, 5, 6, and 8, as Shown by Pressure Test and by Coloration

Variety	Picking[2]	Pressure test (pounds) of lots at intervals[1]					Degree of color[3]
		Time picked	6 days later		12 days later		
			Held moist	Held dry	Held moist	Held dry	
Jonathan	EM	16.5	15.9	14.6	14.9	14.9	2 +
	PM	15.0	15.3	15.3	12.8	13.1	3
	LM	13.7	13.1	13.1	12.0	10.6	—4
	EL	12.6	———	———	9.7	10.3	3 +
Delicious	EM	17.1	16.1	16.6	15.3	13.3	—3
	PM	15.6	14.8	16.2	13.1	12.0	3
	LM	14.3	13.5	13.9	14.1	14.5	3 +
	EM	21.0	18.3	19.1	15.5	16.2	—3
Winesap	PM	17.4	16.8	16.3	17.0	17.2	3
	LM	15.6	17.5	17.1	17.4	17.4	3 +

[1] Average of 30 tests on 10 apples in each case by method of Magness **et al.** (47).

[2] EM = Early-mature ; PM = Prime-mature ; LM = Late-mature ; EL = Extra-late.

[3] Determined on 10 apples by test of Magness **et al.** (47).

was nearly as high as under the conditions of rain which it simulated ; the object was to determine the relation of the frequent fall rains on fruit held in the orchard in picking boxes. Portions of each lot of fruit were packed immediately after the initial treatment, after six days, and after 12 days. Thermograph records were kept of the temperatures through-out the test. The data on the amount of decay were taken on January 17-20, 1932.

The Delicious apples were obtained from five adjacent trees in a block of the Experiment Station orchard with the same cultural treatment as the Jonathans, except that they were sprayed with lead arsenate alone. The early-, prime-, and late-maturity pickings were made September 16, September 24, and October 6, 1931, respectively. The pressure and color tests of the different portions of the Delicious series are given in Table 2. The data on the amount of decay were taken on January 20-29, 1932.

The Winesap apples were obtained from four adjacent trees in a block of the Experiment Station orchard with the same cultural treatment as the Jonathan series, except that they were sprayed with lead arsenate, and a lime-sulphur spray was applied on April 14. The early-, prime-, and late-maturity pickings were made October 1, 16, and 26, respec-

tively. The pressure and color tests of the different portions of the Winesap series are given in Table 2. The data on the amount of decay were taken on February 12-19, 1932.

Time of Picking. A progressive slight increase of the percentage of lenticel infections and (with one exception) of the total blue-mold decay with the increased time the fruit was allowed to remain on the tree is shown in Table 3. The limited data on extra-late picking suggests that there may be a point in maturity beyond which the number of lenticels susceptible to penetration rapidly increases.

Table 3. Percentage of Lenticel Infections and Total Blue-mold Decay in Fruit Picked at Different Stages of Maturity and Dipped in a Spore Suspension. Wenatchee, 1931

Variety	Apples	Maturity			
		Early	Prime	Late	Extra late
Jonathan	Number treated	876	864 ·	751	375
	Per cent blue mold	5.0	10.8	8.0	30.9
	Lenticel infections	1.6	4.4	5.3	25.9
	Probable L. I.	1.9	3.0	1.7	1.1
Delicious	Number treated	674	729	644	
	Per cent blue mold	2.8	4.4	6.7	——
	Lenticel infections	0.3	0.7	4.3	
	Probable L. I.	0.9	1.4	0.8	
Winesap	Number treated	927	912	377	
	Per cent blue mold	0.2	1.0	1.6	
	Lenticel infections	0.2	0.5	1.1	——
	Probable L. I.	0	0.1	0.3	

Time Held in Orchard Previous to Packing, and Moisture Conditions during That Period. The figures of Table 4 show no consistent correlation between the amount of lenticel infection or total blue-mold decay and the length of time the fruit is held in the orchard, but suggest an inverse relationship. The explanation is thought to be that cutinization of the lenticels proceeds better at the orchard temperatures than in cold storage. The average percentage of decay in Jonathan and Delicious apples held for a time was somewhat lower than for fruit stored at once, while the Winesaps stored at once had slightly less decay than fruit held in the orchard. Since the temperature to which the fruit was subjected was considerably lower in the case of later pickings, as indicated by the thermograph records, it was thought that the failure of the Winesaps to show

[1] Exclusive of extra-late picking.

Table 4. Percentage of Lenticel Infections and Total Blue-mold Decay in Fruit Dipped in a Spore Suspension and Held for Various Periods before Storage. Wenatchee, 1931

Variety	Apples	Not held	Held for 6 days	Held for 12 days	Avg. for fruit held
Jonathan[1]	Number treated	839	826	826	1652
	Per cent blue mold	9.5	7.4	6.8	7.1
	Lenticel infections	4.4	4.1	2.5	3.3
	Probable L. I.	2.7	2.1	1.9	2.0
Delicious	Number treated	601	665	781	1446
	Per cent blue mold	5.3	3.6	4.9	4.3
	Lenticel infections	2.8	1.2	1.3	1.2
	Probable L. I.	1.2	0.5	1.4	1.0
Winesap	Number treated	690	759	767	1526
	Per cent blue mold	0.6	1.1	0.7	0.9
	Lenticel infections	0.3	0.7	0.5	0.6
	Probable L. I.	0	0.3	0	0.1

[1] Exclusive of extra-late picking.

Table 5. Percentage of Soft Scald in Jonathan Apples Picked at Different Stages of Maturity and Held Moist or Dry for Varying Periods previous to Storage. Wenatchee, 1931

Time held	Moisture conditions	Maturity			
		Early	Prime	Late	Extra-late
Not held	Moist	0	0.3	0.4	44.0
	Dry	0	1.0	0	——
Held 6 days	Moist	0	14.9	0	——
	Dry	0	39.5	0	——
Held 12 days	Moist	0	0	0	0
	Dry	0.3	1.4	0	0

less decay after being held might have been due to the depressing effect of the lower temperature on the process of cutinization of the lenticels.

These results would suggest that the prevalent belief that holding fruit in the orchard greatly increases decay is exaggerated, and may mean that such treatment merely increases susceptibility to subsequent mechanical injuries in washing and packing operations.

In this connection the data (Table 5) on the occurrence of soft scald in the Jonathan apples of the 1931 series are of interest. This series

showed soft scald largely in fruit of the prime-maturity picking held six days in the orchard before storage and the extra-late picking stored at once. In general, this corroborates the results of Harley and Fisher (31).

Table 6. Percentage of Lenticel Infections and Total Blue-mold Decay in Fruit Dipped in a Spore Suspension and Stored Immediately, or Held Moist or Dry in the Orchard Previous to Storage. Wenatchee, 1931

Variety	Apples	Not held	Held	
			Moist	Dry
Jonathan[1]	Number treated	839	826	826
	Per cent blue mold	9.5	8.6	5.6
	Lenticel infections	4.4	4.2	2.4
	Probable L. I.	2.7	2.4	1.6
Delicious	Number treated	601	727	719
	Per cent blue mold	5.3	4.8	3.8
	Lenticel infections	2.8	1.4	1.1
	Probable L. I.	1.2	1.2	0.7
Winesap	Number treated	690	761	765
	Per cent blue mold	0.6	1.2	0.5
	Lenticel infections	0.3	0.9	0.3
	Probable L. I.	0	0	0.3

[1] Exclusive of extra-late picking.

The data in Tables 6 and 13 show that in every case the fruit held moist had a higher percentage of lenticel infections and total blue-mold decay than fruit held dry. The data in Tables 6 and 13 further indicate that the reduction in the amount of lenticel infection by holding the fruit in the orchard before storage is greater if the fruit is held dry. It is thought that this is due in part to the poorer growth of the fungus, as indicated by its development on the calyx and stem. There are probably two results of delayed storage: (1) a softening of the flesh (see pressure tests on Table 2); (2) a decrease in the number of lenticels susceptible to infection. It is possible that the increase of fungous growth under moist conditions may counteract any beneficial effect delayed storage may have, and for that reason such a practice is not to be recommended without further investigation.

Holding fruit moist also favored the incidence of the gray-mold rot caused by *Botrytis cinerea* Pers., as indicated in Table 7. These data are the summation of all series of the four years' investigations which showed the Botrytis rot.

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Table 7. Number of Jonathan, Delicious, and Winesap Apples with Botrytis Rot in Series Not Held, or Held Moist or Dry

Apples	Not held (Packed wet)	Held moist	Held Dry
Number treated	5,091	6,653	5,887
Number Botrytis rot	1	41	1

[1] Exclusive of extra-late picking.

The comparative susceptibility to infection of the lenticels of apples held moist or dry was investigated by coating fruit from the lot previously discussed (Table 6) with rotten tissue from apples infected with *P. expansum*. The results are given in Table 8. The Jonathans held moist had more lenticel infections and total blue-mold decay than those held dry, as did the Delicious apples if probable lenticel infections are considered.

These findings indicate that holding the fruit in the orchard under dry conditions tends to decrease the susceptibility of the apples to infection at lenticels probably by giving more favorable conditions for drying of the exposed lenticular cells. Apparently no investigations have been made of the relation of the relative humidity of the air during storage to the formation of cuticle and to cutinization of the lenticel basins. Cutinization of plant structures has been shown (43) to be largely a process of dehydration, oxidation, and condensation of fatty acids. It is probable that low relative humidities favor the "closing" of lenticels on the apple fruit, a process which seems to be largely a deposition of cutin in the underlying cells.

Table 8. Percentage of Lenticel Infections and Total Blue-mold Decay in Lots of Apples Held Moist or Dry for 12 Days before Being Coated with Decayed Tissue and Stored. Wenatchee, 1931[1]

Variety and picking	Apples	Held moist	Held dry
Jonathan, prime-maturity	Number treated	55	83
	Per cent blue mold	43.6	24.1
	Lenticel infections	41.8	22.9
	Probable L. I.	0	0
Delicious, early-maturity	Number treated	55	42
	Per cent blue mold	40.0	31.0
	Lenticel infections	21.8	28.6
	Probable L. I.	12.7	2.4

[1] Data taken January 19-29, 1932.

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Another test in 1933 with prime-maturity Delicious apples held in a dry room at a temperature of about 30° C. showed similar results. The fruit was picked on October 11, packed without being washed, and held in cold storage. On November 30 one-half of the lot was moved to a temperature of 30° C. Ten days later the fruit held at 30° C. and the fruit direct from cold storage were coated with tissue of decayed apples and one half of each lot was placed in cold storage and half held in a room at 10-15° C. The results of the examination of the fruit taken from 10-15° C. and from cold storage on November 23 and December 8, respectively, are given in Table 9. Nearly all the apples showed lenticel infections, but there were marked differences in number. As the apples were so completely coated that practically all of the lenticels would be covered,

Table 9. Effect of a Brief Warm Dry Storage in "Closing" Lenticels, as Indicated by the Number of Infections through Them, in Delicious Apples Coated with Tissue of Decayed Apple and Held Subsequently in Cold Storage or at 10-15° C.

| Treatment of fruit | Tempera-ture of storage | Number apples | | Lenticel infections per apple | | |
		Total	With lenticel infection	Maximum	Minimum	Average
Held 10 days at 30° C.	10-15°C.	43	40	21	0	7.4
Direct from cold storage	10-15°C.	42	42	48	1	17.0
Held 10 days at 30° C.	0-2° C.	44	40	37	0	8.3
Direct from cold storage	0-2° C.	43	40	38	0	9.5

the number of infections at these points should be an index of the number of susceptible lenticels per fruit. In fruit held at 10-15° C. following treatment there was a greater reduction in the number of lenticel infections per apple resulting from the heat treatment than in fruit subsequently held in cold storage. It is possible that the return of the "heat-treated" fruit to cold storage affected the structure of the lenticels in some way. A decrease in the number of lenticels susceptible to infection reduces the mathematical chances of infection at such points.

Spore Load of Fruit. Heald *et al.* (36) showed that an increase in the spore load of the washing tank (and thus of fruit passing through it)

would cause a proportional increase in the number of punctures at which decay started in fruit passing through it. As a similar increase in lenticel infections from an increase of the spore load has not been demonstrated, attention should be directed to the summary of results in this connection (Table 10). The greater the spore load of fruit the greater the chance that some of the spores would be in a position to cause infection through a lenticel, and the larger the number of susceptible lenticels the better the chance for spores to be in a favorable position for infection.

Horne and Nitimargi (25) found that the East Malling apples owed their reputation for good keeping qualities and freedom from rot to the low spore load rather than to a high resistance of the fruit. The same condition may prevail to a certain degree for the blue-mold fungus in Washington, but the omnipresence of *P. expansum* and the equalizing of contamination in the washing process limit its operation.

Table 10. Summary of Data on Percentage of Lenticel Infection and Total Blue-mold Decay in Various Lots of Jonathan, Delicious, and Winesap Apples Dipped in Water or in a Spore Suspension

Treatment	Lot of fruit	Number apples	Per cent blue-mold decay		
			Total	Lenticel infections	
				Certain	Probable
Dipped in	Table 1	2,076	7.0	3.8	0.9
spore	Tables 3, 4, 6, and 8	7,129	5.9	3.3	1.2
suspension	Table 12	900	3.2	0	1.2
	Table 15	1,060	7.5	0	1.2
	Average	11,165	6.1	2.8	1.1
Dipped in	Table 1	1,772	4.5	1.7	1.2
water	Tables 3, 4, 6, and 8	6,752	0.7	0.3	0.1
	Table 12	888	0.6	0	0
	Table 15	898	1.8	0.1	0.2
	Average	10,310	1.4	0.5	0.3

Varietal Relations. The varietal susceptibility of apples to infection by blue mold has received little attention. Machacek (45) reported the variety Russet as apparently "immune to outside infection." Schneider-Orelli (63) and Welsh (70) have given some attention to the rate of advance of blue-mold decay in different varieties. The aggregate amount of lenticel infection and total blue-mold decay in the three principal varieties grown in Washington handled under the several conditions of

the 1931 series and other tests should give a fair index of their susceptibility (Table 11). In general, this summary of over 19,000 apples subjected to several different treatments shows the decreasing order of

Table 11. Summary of Data on Percentage of Lenticel Infection and Total Blue-mold Decay in Various Lots of Apples[1]

Variety	Lot of Fruit	Number apples	Per cent blue-mold decay		
			Total	Lenticel infections	
				Certain	Probable
Jonathan	Tables 3, 4, 6, and 8	4,993	4.4	2.0	1.2
	Table 16	463	10.2	4.1	1.3
Delicious	Tables 3, 4, 6, and 8	4,066	2.7	1.0	0.6
	Table 14	1,192	19.6	6.4	2.8
	Table 15	856	6.3	0	0.5
	Table 16	288	6.9	0	1.7
Winesap	Tables 3, 4, 6, and 8	4,447	0.5	0.3	—0.1
	Table 14	1,645	1.9	0.2	0.8
	Table 15	1,102	3.7	—0.1	0.1
	Table 16	288	10.1	0	0.7

[1] Only fruit of the three varieties from the same lot (i. e. handled in the same way) are strictly comparable. Both the fruit dipped in spore suspensions and in water are included.

susceptibility to infection at lenticels and to total blue-mold decay to be: (1) Jonathan; (2) Delicious; (3) Winesap. However, under certain conditions some lots of less susceptible varieties may show more lenticel infections and total blue-mold decay than the more susceptible varieties. The susceptibility of these varieties, as indicated by the rate of radial advance of wound infections, is being investigated to determine the rôle of internal factors in resistance to lenticel invasion.

Washing Operations. The washing operations, if correctly carried out, probably do not greatly affect the number of lenticel infections by blue mold. There has been no increase in blue-mold decay since the inauguration of the washing program, according to available data (4, 36, 58, 65). The writers (4) found about the same number of lenticel infections by blue mold in random samples of commercial washed fruit and certain lots of unwashed fruit in experimental series. Hartman (32) found the "pinhole" rot (i.e. blue-mold lenticel infections) of Winter Nelis pears occurring in both washed and unwashed fruit.

Arsenical Residue. It was thought that the arsenical residue remaining on the fruit in the 1931 series might tend to inhibit the growth of the organism and reduce the amount of infection in that way. For that reason a study of this factor was made in 1932.

Winesap apples were obtained from a Selah orchard (p. 9, lot 3), the fruit from which in previous years had developed many lenticel infections. Half of the lot was washed in unheated 1.5 per cent HCl solution in a commercial packing plant, and half was unwashed. Each of these lots was again divided, half being dipped in a spore suspension and half not treated. The results (Table 12) would indicate that arsenical residues do not reduce the amount of decay caused by *P. expansum* in fruit stored at once. There is even a suggestion that washing might reduce the amount of such decay under some conditions. Possibly the washing tank, as a result of relatively low contamination of fungous spores, reduced the spore load of the apples that had been dipped in a spore suspension, and increased it for fruit that had not been inoculated

Table 12. Percentage of Lenticel Infections and Total Blue-mold Decay in Unwashed and Washed Lots of Winesap Apples, Showing the Effect of Arsenical Residue on Infection. Selah, 1932[1]

Treatment of fruit	Inoculum	Number apples	Per cent blue-mold decay		
			Total	Lenticel infections	
				Certain	Probable
Unwashed	Dipped in spore suspension	450	3.8	0	0.9
	Not dipped in spore suspension	438	0	0	0
Washed in unheated 1½% HCl	Dipped in spore suspension	450	2.7	0	1.6
	Not dipped in spore suspension	450	1.1	0	0

[1]Treated and packed October 11-12, 1932; data taken May 5, 1933.

in this way. Such a condition would be in line with the conclusion (36, 40) that the washing tank is an effective equalizer of contamination of fruit. However, the data (Table 13) on another lot of apples from the Experiment Station orchard at Wenatchee (from lot represented by Tables 3, 4, 6, and 8) showed that fruit held dry and washed just previous to storage had more lenticel infections and total blue-mold decay than that which was unwashed. In light of the recent work of Thom and Raper

Table 13. Percentage of Lenticel Infections and Total Blue-mold Decay in Washed and Unwashed Lots of Winesap Apples Held Moist or Dry. Wenatchee, 1931[1]

Treatment before packing			Number apples	Per cent blue-mold decay		
				Total	Lenticel infections	
First	Second	Third			Certain	Probable
Held 12 days moist	Dipped in spore suspension	Not washed	685	4.4	2.5	0.6
Held 12 days dry	Dipped in spore suspension	Not washed	696	1.6	0.3	0.1
Held 12 days dry	Dipped in spore suspension	Washed in HCl	320	4.4	3.1	0.3
Washed in HCl	Dipped in spore suspension	Held 12 days dry	300	5.7	4.0	1.3

[1] Late-maturity picking, October 26, 1931. Data taken February 19, and March 14, 1932.

(68), showing that *P. expansum* is "arsenic-tolerant" and able to grow in Czapek's solution agar plus arsenic trioxide, it would seem probable that the arsenical residue would not inhibit infection at lenticels.

Time of Washing. On account of the fact that certain commercial lots of fruit that had been held in cold storage for considerable periods before being washed in heated cleaning solutions showed an abundance of lenticel infections, an experiment was conducted in 1932 on this method of handling. Delicious and Winesap apples were obtained from the Experiment Station orchard in Wenatchee on September 29 and October 17, respectively. The fruit was dipped in a spore suspension at once and half of it washed in HCl or sodium carbonate in the Washington Experimental Fruit Washer. The remaining half was stored at 0° C. until November 6, when it was removed and washed in HCl or sodium carbonate in the same machine. These lots of fruit were packed and held in cold storage until data were taken. The results (Table 14) show that, except in one series, the delayed washing increased both the amount of lenticel infection and the total blue-mold decay. Winesap, as would be expected, was better able to withstand these high temperatures than Delicious. The limited data on fruit washed at 49° C. suggest that this high temperature of the cleaning solution is likely to give more lenticel infections and total decay than fruit washed at 43° C. This confirms the findings of various workers (21, 37) that bleaching and lowering of keeping quality result

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Table 14. Percentage of Lenticel Infections and Total Blue-mold Decay in Fruit Washed after Picking or after a Period of Cold Storage. Wenatchee, 1932

Variety	Temper-ture of washing solution	Apples	Washed in 1½% HCl		Washed in sodium carbonate (¾ lb. per gal.)	
			At once	After storage	At once	After storage
Delicious[1]	43° C.[3]	Number treated	210	416	186	380
		Per cent blue mold	15.7	23.1	9.7	22.9
		Lenticel infections	2.9	8.2	2.2	8.4
		Probable L. I.	2.4	4.3	0.5	2.4
Winesap[2]	43° C.[3]	Number treated	286	542	250	567
		Per cent blue mold	1.7	0.9	0.8	3.4
		Lenticel infections	0.7	0	0	0.4
		Probable L. I.	0.7	0.6	0.4	1.2
	49° C.[4]	Number treated	125	282	138	293
		Per cent blue mold	4.0	8.2	1.4	6.5
		Lenticel infections	0	2.1	0.7	1.4
		Probable L. I.	0	2.4	0.7	4.1

[1] Data taken on January 3-4, and on February 21, 24, 1933.

[2] Data taken on May 2, 1933.

[3] Apples dipped in a suspension of blue-mold spores.

[4] Washing tank contaminated by placing several rotten apples, on which the fungus was sporulating, in it.

from cleaning at such high temperatures. It is thought that the sudden change of temperature may rupture the cutinized layers of the lenticels by expansion and contraction. In some lots of fruit the higher temperatures cause noticeable splitting of the skin, starting at lenticels; probably this is a more severe manifestation of the increased amount of decay resulting from washing in heated solutions following storage at 0° C., as shown above. It made little difference in the amount of decay whether acid or soda ash were used in this belated washing.

A further example of the resultant increase of lenticel infection from such high-temperature injury was shown in a lot of Winesap apples in the 1932 Wenatchee crop. During the commercial operations the temperature of the trisodium phosphate cleaner (75 pounds per 100 gallons) rose slightly for a short period on October 22, 1932, but did not reach 49° C. Certain portions of the fruit assumed an inconspicuous dull appearance at that time, but the fruit regained its normal appearance with the development of wax in storage. The fruit was dipped at once in a spore suspension and placed in cold storage. A summary of the

examinations of May 2 and July 25, 1933 showed that 8.8 per cent of the 125 apples had clear-cut lenticel infections and 16.0 per cent more had infections probably of lenticel origin; a total of 44.0 per cent of the fruit showed blue-mold decay. Another lot of Fancy Arkansas Black apples from Yakima examined on February 26, 1932 showed marked bleaching and scalding from the washing process; 33.3 per cent of the 250 apples had infections at the lenticels, and a total of 42.4 per cent of the apples showed blue-mold decay.

Examination[1] of commercial fruit in Yakima in January, February, and March, 1934, has given further evidence of the danger of washing fruit at high temperatures. One packing house had many lots of fruit washed in sodium silicate at 54 to 55.5° C. which showed high percentages of lenticel infections and total blue-mold decay.

Moisture Conditions during Growth. Reed and Crabill (57) and Heald (35) reported a visible splitting of the lenticels following a sudden supply of water after a period of dryness. Haylett (34) immersed apples in water, and found that the more turgid the fruit the greater was the permeability of the lenticels, as indicated by dye tests. Adam (1) found that pears from irrigated plots had 3 and 20 per cent decay (unspecified cause), while those from unirrigated plots had 14 and 35 per cent decay, in wrapped and unwrapped fruit, respectively, after being held at 1° C.

Table 15. Percentage of Lenticel Infections and Total Blue-mold Decay in Lots of Fruit from Trees on Irrigation Plots. Fruit Dipped in Spore Suspension. Prosser, 1932

Amount of irrigation per year	Variety	Number apples	Per cent blue-mold decay		
			Total	Lenticel infections	
				Certain	Probable
Light (30″ of water)[1]	Delicious[3]	214	14.5	0	0.9
	Winesap[4]	313	8.3	0	2.9
Heavy (60″ of water)[2]	Delicious[3]	216	10.2	0	0.9
	Winesap[4]	317	0	0	0

[1] Applied for 9 hours every 15 days on Delicious, and for 18 hours every 30 days on Winesap; totals 30 inches per year, exclusive of rainfall.

[2] Applied for 18 hours every 15 days on Delicious, and for 18 hours every 30 days on Winesap; totals 60 inches per year, exclusive of rainfall.

[3] Picked September 27, 1932; data taken on January 3, 1933.

[4] Picked October 10, 1932; data taken on May 5, 1933.

[1] Unpublished data collected by Paul Allen, graduate student in the Department of Plant Pathology.

The theory of Devaux (20) previously mentioned, that moisture conditions greatly influence the condition of the lenticels led to an investigation of this factor in 1932. Unwashed Winesap and Delicious apples were used from the irrigation plots in the Irrigation Branch Station orchard at Prosser, half being dipped in a spore suspension and packed wet and the other half packed dry. The fruit was held in cold storage until examined. The results (Table 15) do not allow any conclusion concerning the relation of the amount of irrigation water to the susceptibility of lenticels to infection, but do show a marked increase of the total blue-mold decay in fruit from plots with light irrigation, verifying Adam's results with unspecified organisms.

Abrasion and Bruising of Fruit. Kidd and Beaumont (42) found the spores of various fungi scattered over the surface of the apples and about 31 per cent of them in the lenticel basins. Treatment with mercuric chloride killed all of the spores except those in the lenticel basins. Such a location would favor the ready penetration of the lenticel upon germination of the spores. Heald *et al.* (36) found that the dry-wiping process of arsenical-residue removal of 1926 spread the spores over the fruit, and thought that it rubbed them into the wax, lenticels, russet areas, and cracks. However, the amount of decay was not markedly increased.

During the many handling operations from picking to packing the fruit is rubbed against other apples, picking boxes, conveyer belts and rollers, grading table, sizing machine, packers' gloves, etc. This continued abrasion gives an excellent opportunity for spores to get into lenticels and so be in an advantageous position for infection. An investigation was made on this point in 1932. The Jonathan and Winesap apples were obtained from an orchard near Selah (p. 9, lot 3), the fruit from which in previous years had developed many lenticel infections, and the Delicious were obtained from another Selah orchard. One portion of the fruit of each variety was rubbed briskly with packers' gloves into which a large volume of dry spores of *P. expansum* had been placed; part of this lot was packed at once and part packed after washing. Another portion was washed in an unheated HCl solution before being rubbed. All lots were held in cold storage. The results (Table 16) indicate that abrasion does not greatly affect the susceptibility of lenticels. While the data are inconclusive, it would appear that abrasion alone does not greatly affect the number of lenticel infections. Fruit washed previous to rubbing, and to a lesser extent, fruit washed after rubbing, showed more decay than unwashed fruit handled in the same way. The washing process probably carried the spores into the lenticels and supplied the moisture for germination. The marked increase of calyx infections in the rubbed and washed lots cannot be explained without further work.

During the last two years occasional apples have been seen that had one to many lenticel infections on the margins of bruises and of flattened

Table 16. Percentage of Lenticel Infections and Total Blue-mold Decay in Lots of Fruit Rubbed with Gloves Coated with Spores. Selah, 1932[1]

Treatment of fruit	Variety	Number apples	Per cent blue-mold decay			
			Total	Calyx	Lenticel infection	
					Certain	Probable
Untreated	Jonathan	43	4.7	0	2.3	2.3
	Winesap	438	0	0	0	0
Washed[2]	Winesap	450	1.1	0	0	0
Rubbed with spores[3]	Jonathan	163	2.5	0	0.6	0
	Delicious	138	8.0	2.9	0	2.9
	Winesap	150	8.0	3.3	0	0
Rubbed with spores[3] and washed[2]	Jonathan	300	14.3	0	6.0	.02
	Delicious	150	6.0	4.7	0	0.7
	Winesap	138	12.3	5.8	0	1.4
Washed[2] and rubbed with spores[3]	Delicious	138	35.5	28.2	2.9	4.3
	Winesap	150	11.3	8.0	0	3.3

[1] Jonathan treated and packed September 22, Winesap on October 12, and Delicious on October 13. Data on Jonathans taken December 21, 1932; figures for other varieties are totals of inspections on January 4-5, and May 2, 5, 1933.

[2] Washed in unheated 1½ per cent HCl.

[3] Packers' gloves heavily coated with spores of **P. expansum** (from cultures started May 15); each apple vigorously rubbed.

areas produced by contact with the box or with other fruit. It is thought that localized pressure may rupture the layer of cutinized cells in the lenticel basins and thus increase susceptibility.

Fruit Size. Examination of commercial lots of fruit (4) and four years' experimental work have failed to show any correlation of fruit size and the amount of lenticel infection. Size is affected by so many conditions of the environment that specific investigations of the correlation of this factor to lenticel infections have not been made.

Fertilizer Treatment of Orchard. The effect of fertilizer treatment of apple trees on the incidence of decay has been investigated largely from the standpoint of the rate of advance of the decayed area produced by weak parasites inoculated into the fruit (25, 26, 27, 38). Gourley and Hopkins (28) showed that the amount of decay (unspecified cause) was not correlated with fertilizer treatment. Onslow, Kidd, and West (26)

found that apples from eight soils of low nitrogen-content had less decay (unspecified cause) than fruit from plots of high nitrogen.

The effect of applications of nitrogen, potash, phosphorus, or their various combinations on the susceptibility of lenticels to infection by *P. expansum* was studied in a preliminary test in 1933. Prime-maturity Jonathan apples were obtained from the Experiment Station fertilizer plots in East Wenatchee. The fertilizers used were ammonium sulphate (N), superphosphate (P), and potassium chloride (K). Six pounds of superphosphate were used annually per tree and five pounds of each of the other components. The applications began in 1927 with the exception of NP and NK plots, which were started in 1928. The check plot had received no fertilizers since 1927.

The fruit was packed and held in cold storage in Pullman until December 17, when it was removed and coated with the tissue of apples completely decayed by *P. expansum*. The examination of the fruit on February 1, 1934 gave the data shown in Table 17.

Table 17. Number of Lenticel Infections in Jonathan Apples from Trees in the East Wenatchee Fertilizer Plots, Resulting from Coating Fruit with Decayed Tissue. 1933

Fruit from fertilizer plot	Number apples	Lenticel infections per apple		
		Maximum	Minimum	Average
N	40	23	0	7.4
P	35	21	0	6.9
K	42	16	0	5.8
NP	36	29	3	12.4
NK	32	20	0	6.9
PK	40	38	0	8.5
NPK	39	28	1	10.5
Check	39	24	0	7.4
Average, nitrogen series	147	—	–	9.3
Average, non-nitrogen series	156	—	–	7.1

These limited data show no constant relationship between the application of any of the three salts to the various plots and the susceptibility of the fruit to infection at lenticels. A number of lenticels susceptible to infection occurred on fruit from all of the fertilizer plots, but the average was greater for series receiving nitrogen than for non-nitrogen plots.

The results of tests to be published later showed that the radial advance of *P. expansum* in the tissue of the apple was generally greater in fruit from fertilizer plots than from check plots, and in fruit from

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plots receiving nitrogen than from non-nitrogen plots. However, the rate of radial advance did not vary consistently with any given type of fertilizer used.

While fertilizers probably are important in determining the susceptibility of fruit to less virulent parasites, their effect in altering susceptibility to *P. expansum* would seem to be slight. This factor is certainly not sufficiently important to influence materially the incidence of blue-mold decay.

Indices of Susceptibility of Lenticels to Infection

Magness (46) used the penetration of an aqueous solution of methylene blue after a 15 to 30 minute submersion as an index of picking maturity and the number of open lenticels in Bartlett pears. Overholser and Latimer (53) found that the discoloration produced by the penetration of ammonia fumes through lenticels of pears served as an index of the degree of their openness and number.

Kidd and Beaumont (42) measured the porosity of lenticels by the rapidity with which air was given off when apples were submerged in water under reduced pressure. The open lenticels showed up better when apples were slightly evacuated under water containing methylene blue. Haylett (34) employed the submersion of apples in eosin dye under 30 inches of vacuum as an indication of their permeability; in other tests nigrosin and India ink were employed at normal atmospheric pressures.

A similar process for the quantitative and qualitative determination of the "openness" of lenticels has been employed by Clements[1] during the past three years in work on the histology of the lenticels of the apple. Fruit is submerged in an aqueous solution of methylene blue under normal atmospheric pressure for two to three days, penetration being facilitated by the normal diurnal temperature variations of the laboratory. The high temperatures at night in conjunction with the lower temperatures during the day supply a slight expansion and contraction of the fruit which condition gives the same result as reduced pressures with less likelihood of rupturing the lenticels.

A test was made to obtain positive evidence of the relation of the open type indicated by the dye test and susceptibility as evidenced by lenticel infection when coated with decayed tissue. Delicious apples were obtained from a ranch near Selah (lot 5, Table 1) and held in cold storage until December 22, when they were placed in the dye for 24 hours. The fruit was then rinsed in water and coated with decayed apple tissue. After being held for nine days at 10-15° C. the data (Table 18) were taken. The number of decayed areas centered on lenticels of each type

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[1] Unpublished investigatoins of Dr. H. F Clements in the Department of Botany.

Table 18. Blue-mold Infections in Delicious Apples Dipped in Dye to Indicate the Type of Lenticels and then Coated with Tissue of Decayed Apple, Showing the Correlation of the Dye Test and Susceptibility to Lenticel Infections

Apple number	Infections at lenticels of			Infections of uncertain origin	Total infections exclusive of uncertain
	Open type	Partly-open type	Closed type		
1	13	0	0	0	14
2	9	0	0	2	9
3	11	0	0	12	12
4	23	0	0	many	23
5	1	1	1	0	4
6	21	1	0	many	23
7	16	0	0	0	17
8	12	1	0	many	14
9	8	1	0	0	10
10	7	0	0	3	8
11	6	0	0	0	7
12	0	0	0	many	0
13	9	0	0	0	10
14	2	2	1	0	6
15	6	1	0	0	7
16	0	0	0	0	0
17	5	1	0	many	7
18	13	0	0	0	15
19	19	0	0	0	20
20	19	0	0	0	19
21	10	0	0	few	11
22	31	0	0	0	32
23	21	1	0	many	24
24	19	1	1	many	21
25	15	0	0	0	15
26	4	0	2	0	6
27	0	0	0	0	1
28	27	0	1	many	30
29	10	0	0	0	12
30	10	0	0	0	11
31	8	2	0	0	11
32	8	0	0	0	8
33	14	0	0	0	16
34	10	4	1	0	16
35	2	0	0	0	2
36	5	0	0	many	6
37	10	0	0	0	11
38	5	1	0	few	7
39	31	0	0	0	32
40	9	0	0	few	10
41	17	0	0	0	18
42	11	1	0	0	13
43	19	0	0	0	20
44	8	0	0	0	10
45	10	0	0	0	10
46	14	0	1	0	15
47	13	0	0	0	13
48	12	0	0	0	13
49	27	0	0	0	27
50	18	0	0	0	19
51	7	0	0	many	8
52	0	0	0	0	0
Total	605	18	8	-----	672
Average per apple	11.6	0.3	0.2	-----	12.9

(as indicated by the dye) are given; infections of uncertain origin were those which developed on small scalded areas.

It is to be noted that 605 out of 672 infections of identifiable origin were at lenticels of the open type. Histological studies by Clements have not shown alteration of lenticels from the dye treatment.

The accuracy of the test made by coating the fruit with decayed tissue might appear to be questionable. This method is believed to be a fair one for three reasons: (1) histological studies have shown open lenticels (of the dye or decayed-tissue test) to be those in which the uncutinized tissue is exposed, a condition that would favor infection; (2) the action of the enzymes of the decayed tissue on the lenticels would probably be limited to uncutinized tissue, because no enzymes are known to act on cutin or suberin; (3) about the same relationships are shown in lots (e.g. Tables 6 and 8) dipped in spore suspension and those coated with decayed tissue, the difference being one of degree rather than type.

The decayed-tissue test would appear to give a useful index of the number of uncutinized (open) lenticels and thus of the relative suscep-tibility of a given lot of fruit. In view of the close correlation of the dye and decayed-tissue tests it would seem that the methylene blue test will also give a useful index of susceptibility of the lenticels to infection. These indices would be of value in indicating the susceptibility to infec-tion of the lenticels in a given lot of fruit and thus whether the applica-tion of control measures is justifiable. However, further investigation is necessary before they could be used commercially.

Spore Germination in the Process of Infection at Lenticels

As spores of *P. expansum* do not germinate well in plain water (5, 27, 71), but do germinate well in moisture on the apple, it is apparent that a stimulus exists. This may be: (1) a stimulus from proximity to the apple, possibly from its volatile esters; (2) juice from adjacent rotted fruit; (3) exosmosis of nutrients through lenticels to the moisture collected on the surface of the fruit; or (4) acidity arising in the moisture on the surface of the apple.

In preliminary tests, Brown (15) found a marked stimulation of germination of spores of *P. glaucum* Link (probably *P. expansum*) when placed in damp chambers in which apples were present. This was thought to be the result of some volatile product of the fruit. This conclusion has been fully verified a number of times by the writers, the agent of stimulation apparently arising from the flesh of the fruit. Spores of *P. expansum* in sterile distilled water grow readily when near Delicious, Jonathan, or Winesap apples and, after a short period of development, will form short chains of spores typical for the genus. This form of stimulus alone would seem to be sufficient to allow infection of lenticels

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from spores in advantageous positions without any of the other three conditions being fulfilled.

The relation of the juice of rotten tissue to decay will be discussed in relation to its effect on the lenticels (p. 35). Such a liquid also supplies sufficient nutrient material for the fungus to establish itself; while this action is of less consequence than the effect of the enzymes on the lenticels it cannot be disregarded. From a commercial standpoint this stimulus is too infrequent to account for the germination of the spores.

The exosmosis of nutrients through lenticels has been emphasized by Kidd (24) as the "dominant factor controlling fungal invasion." It was thought that "the exosmosis of nutrient material must increase with the age of the apple, or that the percentage of lenticels through which exosmosis takes place becomes proportionally greater." The accompanying theory of "local senescence" of lenticels as the source of the nutrients seems to the writers to be unnecessary for *P. expansum.* While it is not questioned that small amounts of sugars and acids will move by exosmosis through the cellulose walls of the cells of an open lenticel into the moisture of the lenticel basin, the actual presence of reducing sugars could not be demonstrated by tests with Fehling's solution either in moisture from the surface of the apple or in water in which 10 Delicious apples (Table 21) had stood for 27 days. Moisture treated with sulphuric acid previous to testing with Fehling's solution also gave a negative test for reducing sugars. Kidd and Beaumont (42) did not show the presence of sugars in such moisture, but did show an increase of electrolytes by measurement of the electrical conductivity. The various fungous spores would germinate in small tubes of water over lenticels and not in checks placed between lenticels. It is to be noted that the presence of malic acid in the greatest concentrations found in apples would not prevent the development of the fungus. (8, 9).

Hartman, Childs, and Robinson (33) found that "water in the calyces of apples may become acid," as indicated by its ability to dissolve arsenic trioxide. This phenomenon might well be involved in the exosmosis of electrolytes mentioned above. Machacek (45) found that germination of *P. expansum* spores in distilled water was greatest in that which was acidified. Webb (69), after studying various fungi (including Penicillia), stated that "Germination is a process which is strikingly supported by conditions of active acidity." This fourth stimulus to germination might be of considerable importance in some cases, but has not been investigated.

Growth from Adjacent Decayed Apple

The reports of infection of blue mold from rotted fruit have already been mentioned under Literature Review. Tests with fruit coated with tissue of decayed apples showed that these statements were correct. Accordingly an investigation of the explanation of this phenomenon was

Table 19. The Effect of Decayed Apple Tissue on the Amount of Infection through the Lenticels of Winesap Apples Washed in Unheated HCl. Wenatchee, 1931[1]

Time held before packed and stored	Apples	Treatment of fruit previous to packing moist							
		Dipped in suspension of spores in		Coated with		Dipped in cider	Held moist 13 days; rinsed in aqueous suspension of spores		
		Water	Cider	Decayed apple tissue plus spores	Heated[2] decayed apple tissue plus spores		Coated with decayed apple tissue	Coated with heated[2] decayed apple tissue	Held moist 13 days; dipped in aqueous suspension of spores
Not held after treatment	Number treated	75	75	75	75	94	83	79	107
	Per cent blue mold	0	1.3	68.0	28.0	3.2	88.0	8.9	1.9
	Lenticel infections	0	1.3	37.3	18.7	2.1	42.2	2.5	1.9
	Probable L. I.	0	0	2.7	1.3	0	0	1.3	0
	Calyx	0	0	25.3	5.3	1.1	44.6	5.1	0
Held moist 13 days after treatment	Number treated	113	105	94	110				
	Per cent blue mold	1.8	5.7	88.3	20.9				
	Lenticel infections	0.9	1.9	40.4	7.3				
	Probable L. I.	0	1.0	11.7	0				
	Calyx	0	0	48.9	13.6				

[1] Series started on October 20, 1931. Extra Fancy Winesap apples used. Data taken January 9 and February 12, 1932.
[2] Tissue of rotted apple heated to boiling to inactivate the enzymes present.

begun in 1931. Winesap apples (same lot as in Tables 3, 4, 6 and 8) from the Experiment Station orchard in Wenatchee were handled in the ways shown in Table 19.

Fruit held 13 days moist was kept in the orchard under the canvas cover already mentioned. Fresh cider was used. The decayed apple tissue was taken from partially rotted fruit and was mixed into a thin paste before using.

The fact that the fruit dipped in a spore suspension in cider showed but few more lenticel infections than fruit dipped in a similar suspension in water would call into question the application to *P. expansum* of the conclusion of Kidd (24) that "The presence or absence of suitable nutrition for the germination and development of the fungal spores in the lenticels appears to be the dominant factor controlling fungal invasion." The fruit coated with the tissue of decayed apples and packed at once or held moist for 13 days after treatment had many more lenticel infections than fruit coated with similar decayed tissue heated to boiling and subsequently cooled. Since both the unheated and heated decayed tissue had an abundance of blue-mold spores added just before smearing, and since both had the same texture and moisture content, there are but two factors that may explain the reduced infection from the use of the heated tissue. The first is the decreased "vigor of development" of the fungus (42), and the second is the destruction of the enzymes present in the rotted tissue.

Machacek (45) has shown that *P. expansum* spores will germinate and develop very well on medium staled by previous growth of the fungus. As the many viable spores added to the heated tissue would germinate and develop, perhaps even more vigorously than the old hyphae already present in the tissue, the first explanation would not seem to be applicable. Neither is it a "mass action." It is probable that the enzymes present alter the uncutinized cells of the lenticels and increase the chance of infection.

The repeated demonstration of the production of cellulase (17, 59, 61, 62) and pectinase (8, 17, 51, 56) by *P. expansum* strengthens this hypothesis. A comparison of the amount of lenticel infection in fruit coated with decayed tissue and stored at once and the lot coated for only 13 days shows that the action on the lenticels had occurred during the 13-day period. The fruit coated with decayed tissue and held moist for 13 days showed 42.2 per cent lenticel infection, while another lot which was rinsed in water after being coated with heated decayed apple tissue and held 13 days showed but 2.5 per cent of such infections. The latter figure may be taken as the true index of the action of the heated tissue because (1) it was shown that the enzymatic action will occur in the 13-day period, and (2) the higher percentage of lenticel infection in fruit coated with heated decayed tissue and stored can be explained by the subsequent growth and enzyme formation by the germinated spores.

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In summary of this argument we have, then, the infection of some of the lenticels of 2.5 per cent of the fruit resulting from the favorable circumstances of moisture and food supply, and the infection of 37.3 and 42.2 per cent of the apples (in two lots) resulting from the action of the enzymes, moisture, and food supply present. The "vigor of development" stressed by Kidd and Beaumont is comparatively unimportant for this fungus because in the presence of moisture and food supply there is only a slight amount of lenticel infection. The prevalence of calyx infection in this experiment is of interest.

Another test was made in 1932 on this problem (Table 20). While the Jonathans (from the Experiment Station orchard in Wenatchee)

Table 20. The Effect of Decayed Apple Tissue on the Percentage of Infections through the Lenticels of Jonathan Apples. 1932[1]

| Treatment of fruit previous to packing | Number apples | Per cent blue-mold decay | | |
| | | Total | Lenticel infections | |
			Certain	Probable
Dipped in aqueous spore suspension	38	7.9	0	0
Held ½ hour in filtered juice of decayed apple; dried; dipped in spore suspension	72	6.9	0	1.4
Coated with pulp of decayed fruit	62	30.6[2]	9.7	1.6
Coated with pulp of normal fruit	74	0	0	0
Coated with pulp of normal fruit plus aqueous suspension of spores	37	5.4	2.7	0
plus spore suspension and filtered juice of decayed apple	71	18.3	5.6	0
plus filtered juice of decayed apple for 2 days; rinsed in water; dipped in spore suspension	75	18.7	4.0	0

[1] Series started on October 5, 1932, using Extra Fancy Wenatchee Jonathans. Pulp of normal fruit from peeled and ground Jonathans of same lot; pulp of decayed fruit from Winesaps of the 1931 crop. Data taken December 19, 1932.
[2] 16.1 per cent with calyx infections.

used gave only 9.7 per cent lenticel infections when coated with decayed tissue (indicating a low susceptibility), the results are of interest. The results from the lot held for one-half hour in the juice of decayed apples that had been filtered several times to remove the apple tissue and fungous

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mycelium suggested that the action of the enzyme was fairly slow. The fruit coated with ground apple tissue plus juice of decayed apple and held for two days showed that the action on lenticels had occurred by that time. The ground pulp of normal Jonathan apples applied to fruit gave no lenticel infections, but the addition of spores to the ground tissue gave 2.7 per cent of such invasions. Ground tissue plus spores and filtered juice of decayed apples gave 5.6 per cent of the fruit with lenticel infections. In other words, the addition of the enzyme increased the number of lenticel infections over the number found in fruit treated in such a way that the fungus could develop the same enzymes in smaller amounts *in situ*.

Another test was made in 1933 with unwashed prime-maturity Delicious apples from the Experiment Station orchard in Wenatchee (Table 21). The fruit was heavily coated with inoculum by rubbing with dry

Table 21. Number of Blue-mold Lenticel Infections in Delicious Apples Held in the Expressed Juice of Normal or Decayed Apples for 14 Days[1]

Fruit held in	Number apples	Lenticel infections	
		Total number	Average
Juice of decayed fruit	10	36	3.6
Fresh cider	13	14	1.1
Water[2]	10	0	0

[1] Fruit heavily coated with dry spores of **P. expansum** previous to being held for 14 days in stated solutions. Test started November 3, 1933.
[2] No infections by November 29, 1933.

spores of *P. expansum* from cultures. The fruit was then placed in pans of the given liquids and held at room temperatures for 14 days. The evidence of Table 21 is thought to indicate further that the enzymes present materially assist infection, and that the "vigor of development" of the fungus in the cider (which became fermented) was not as important a factor as the action of the enzymes on the host, assuming that other physical characters of the liquids were comparable.

The final proof, by isolation and purification of the enzymes, of the theory that such catalysts are involved in the increase in the number of lenticel infections when fruit is in contact with decayed tissue has not been made.

Green (29) investigated the contact infections of oranges by *Penicillium digitatum* Sacc. and *P. italicum* Wehmer. She found that one of the factors influencing such infections was "The activity of the resistance-

destroying system which is produced by Penicillium on a mouldy orange but not in synthetic media." A spore suspension of orange juice painted on the fruit gave frequent infections when held at 25° C. under humid conditions, but a similar test with Dox' agar gave uniformly negative results. Various culture media plus spores did not promote infection of wounds, whereas similar suspensions in orange juice or in various organic and inorganic acids (which did not support growth) aided infection. The "system" was not a matter of pH of the environment, however, for acidifying synthetic media previous to smearing on the fruit did not increase infection.

An investigation was made in 1933 to determine whether lenticel infections by *P. expansum* were favored by coating fruit with cultures of the organism grown on two per cent dextrose potato-agar for one month The fruit was treated on January 27, 1933, and data taken on February 27 after being held at 10-15° C. The check (fruit coated with tissue of decayed apple) was also held in a room at 10-15° C., and results were taken on February 20. The results on Delicious apples (Table 22) show that there was an average of 3.0 lenticel infections per apple when coated with the culture and 3.8 when smeared with the tissue of decayed fruit. It is apparent that no "resistance-destroying system" is present in apple tissue decayed by *P. expansum* that is not also found in the culture media, and that there are marked differences in the mechanism of action of *P. expansum, P. digitatum,* and *P. italicum.* The results indicate the production on culture media as well as in host tissue of the enzymes which act on the lenticels.

Occurrence of Lenticel Infections at Cold Storage Temperatures

Schneider-Orelli (64) and Horne (23) found that spores of *P. expansum* placed in wounds in apples germinated and caused decay when held at about 0° C. Brooks and Cooley (11) found that two varieties of apples inoculated with the organism showed no decay after 4 months in cold storage (0° C.), but laboratory tests showed that infection did occur at that temperature. Brooks and Hansford (14) reported slight growth of *P. expansum* on meat at -6° C., better growth at -1° C., and good growth at 2° C.

While it was not definitely known that lenticel infections could occur at 0° C., such a condition was suggested by the variation in size of lesions on fruit in commercial storage and experimental lots late in the season. An investigation of this point was made in 1933 (Table 22). Washed Delicious apples were held at -0.5 to 0° C. for three days to stabilize the temperature, removed and coated at once (January 27, 1933) with tissue from decayed apples, and returned immediately to the electric refrigerator. Two months later (March 28) the fruit was removed and the tissue of decayed apple peeled off; the number of visible lenticel

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Table 22. Number of Lenticel Infections in Washed Delicious Apples Covered with Tissue of Decayed Apple or with Cultures of *P. expansum* and Held at 0° and 10-15° C.

Apple number	Number of lenticel infections in apples held at 0° C.			Number lenticels per apple held at 10-15° C.		
				Covered with decayed-apple tissue		Covered with culture and held moist
	March 28	March 30	April 3	Held dry	Held moist	
1	1	2	2	1	5	2
2	6	7	7	2	10	4
3	1	1	1	4	4	1
4	1	3	5	1	4	3
5	1	2	3	6	4	3
6	4	4	5	4	2	3
7	2	3	4	5	1	5
8	1	1	4	5	4	3
9	1	5	7	6	4	2
10	0	2	3	7	5	4
11	0	1	2	1	3	-
12	0	1	1	1	0	-
13	0	0	1	-	-	-
14	0	0	0	-	-	-
Average per apple	1.3	2.3	3.2	3.6	3.8	3.0

infections increased for six days when held at room temperature. Undoubtedly all of these infections were present in incipient stages when the fruit was removed from the refrigerator, and developed rapidly with the advance of temperature. The same condition has been noted in several other experimental lots of fruit. Extra Fancy Winesap apples, collected[1] in Wenatchee in January, 1934 and shipped to Pullman, showed a decided increase in the number of lenticel infections after being held in the laboratory for a few days. It is probable that this phenomenon explains some of the cases of the sudden appearance of considerable percentages of blue-mold decay in lots of fruit after their removal from storage. This would be in accord with the statement (32) that the "pinhole" rot of Winter Nelis pears "usually makes its appearance shortly after the fruit comes out of cold storage."

The findings of other workers on the ability of the fungus to initiate decay in wounds at 0° C. were verified in another test. Delicious apples were held at 0° C. for three days to stabilize the temperature, then

[1] Collected by G. A. Newton while working on the C. W. S. Apple Rot Survey of the Plant Pathology Department in 1934.

removed, punctured, inoculated, and returned to the refrigerator without delay. The inoculations were made by hypodermic needle into wounds made by the method of Huber (39), a measured amount of a uniform spore suspension being placed in each wound. Of 33 punctures, decay had started in 31 in two months. The maximum, minimum, and average radial diameters of the decayed areas were 52.5, 4.0, and 30.4 mm., respectively. The average size of the decayed areas of the three punctures per apple for the 11 apples used was as follows: 49.2, 47.3, 36, 35.3, 34.3, 29.7, 28, 23.2, 13.2, 11 and 9.7. These measurements show the variation in development to be expected in apples of uniform size, maturity, and history of development, with a definite amount of inoculum in punctures of uniform size, the fruit being held under constant conditions of temperature and humidity. These figures furnish a very large range of variation. However, Gregory and Horne (30) found that the best index of the rapidity of progress of decay in apples was the radial advance of the organism.

The results of these investigations indicate that cold storage reduces decay by retarding the development of the fungus rather than by preventing infection.

DISCUSSION AND CONCLUSIONS

In a field survey grower-lots showing severe losses from lenticel infections every year were found representing one extreme of the importance of the trouble. Intermediate types ranging nearly to the hypothetical polar condition of absence of lenticel infections were also found. The same situation exists for total blue-mold decay.

The grower or buyer must decide whether the losses suffered due to decay in a given lot of fruit over a period of years justify the expense of special control measures. At the present time such a program should be of two types:

(1) General practices (e.g. avoidance of injuries to fruit, sanitation, alteration of cultural practices, etc.) applied to all fruit.

(2) Special practices (e.g. treatment of fruit with a fungicide) applied only to fruit from ranches having a large amount of decay (a) nearly every year or (b) in occasional years.

The two indices of susceptibility of the lenticels presented in this paper are potentially valuable in cases of type 2b above to determine whether special control measures are justifiable in a given year. This phase of the problem is treated in more detail in another place (6).

It is apparent from the data presented that there are three main conditions affecting the incidence of lenticel infections: (1) susceptibility of the lenticels; (2) number of blue-mold spores on the fruit; (3) factors influencing the process of infection.

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The susceptibility to lenticel infection and to total blue-mold decay increased with delayed harvesting of the fruit; there may be a point in maturity at which the number of susceptible lenticels rapidly increases. No consistent effect was shown on the total blue-mold decay or the susceptibility of the lenticels by the time held in the orchard before storage, but there usually was an inverse relationship. However, if the fruit was wet during the period the effect of delayed storage was somewhat reduced; the susceptibility of lenticels, total blue-mold decay, and Botrytis rot were all greater in fruit held moist than in that held dry. Holding the fruit in cold storage for a period previous to washing increased the amount of lenticel infection and total blue-mold decay; limited data suggest that washing at higher temperatures is more damaging in this respect than in moderately heated tanks. Bruises and localized pressure spots seemed to increase susceptibility of the lenticels in that area.

The arsenical residue appeared to be unimportant in inhibiting the development of the fungus at the lenticels. The data obtained on the effect of the total amount of water applied at an invariable rate during the growing season on the susceptibility of lenticels to infection were inconclusive; the total blue-mold decay was greatest in plots with light irrigation. Fruit size in itself is thought to be of no significance in altering the susceptibility of the lenticels to infection, but since it is a result of the action of conditions also affecting susceptibility a correlation might be established. The constant abrasion of fruit in handling operations probably places spores in lenticel basins, but in this test it did not seem to alter the susceptibility of the lenticels to infection. No consistent effect of applications of nitrogen, potash, phosphorus, or their various combinations on the susceptibility of lenticels to infection was evidenced in this work; the average number of lenticel infections per apple in fruit from plots receiving nitrogen was greater than in fruit from non-nitrogen plots.

The three principal apple varieties of the state showed consistent differences in the matter of the susceptibility of the lenticels to infection; in the order of the increasing susceptibility these were Winesap, Delicious, and Jonathan. No immune varieties have been found in incidental study of this problem.

It is evident that the susceptibility of lenticels to infection (i.e. their "openness") is conditioned by a multiplicity of factors of the environment and of the fruit. Several of these have been investigated, and there are doubtless many others as yet undiscovered. The complex interactions of these factors make it improbable that any given one will always result in altered susceptibility. For that reason some of the factors which showed a neutral effect in these studies may later be found to produce measurable effects. Any attempt, therefore, to alter all of the cultural practices bearing on the problem would be hopeless. As this type of control seemed to be decidedly inferior to treatment of the fruit with a sodium hypo-

chlorite rinse (6), further study of these predisposing factors was considered unprofitable from the commercial standpoint.

Extreme differences between entirely comparable apples, both in number of susceptible lenticels and in rate of advance of the decay, indicate the variations to be expected in the internal and external mechanisms of resistance mentioned by Zschokke (73). The problem of evaluating the relative susceptibility to lenticel infection of a given lot is worthy of considerable attention.

It was shown that the greater the load of blue-mold spores per apple the greater are the chances of infection at lenticels. The small percentage of fruit surface represented by lenticel areas reduces the chance that spores would actually get into lenticel basins; this condition is largely overcome by the germination of the spores and the spread of the mycelium over the surface of the fruit. While low contamination of the fruit may in some cases be a limiting factor to the number of blue-mold lenticel infections occurring, the omnipresence of the spores of *P. expansum* makes this condition of less importance than in the cases of many other fungi causing decay.

The mechanism of the growth of the fungus from an adjacent previously rotted apple was studied in detail, as a modification of this phenomenon was used as an index of susceptibility. The "vigor of development" of the fungus is thought never to be a limiting factor to the incidence of lenticel infections by *P. expansum* because of the other three stimuli of germination of spores. As the activity of decayed tissue in increasing infection is lost by heating, and as cellulase and pectinase have been frequently demonstrated for the organism, it is thought that enzymes are the agents involved. The effectiveness of the decayed tissue in increasing the number of lenticel infections was shown to be caused in only a limited degree by the moisture or food supply provided, or to the "mass action" by the fungus; the slight apparent action of these factors might be due to production of enzymes *in situ* by the fungus. As *P. expansum* will infect at lenticels nearly as readily from culture media as from apple tissue, there is no "resistance-destroying system" produced by the organism comparable to that found (29) for the citrus Penicillia.

It is thought that there are four stimuli of germination of the spores of *P. expansum* on the surface of the apple or in the lenticels: (1) volatile products of the apple; (2) the juice from adjacent decayed fruit; (3) the exosmosis of nutrients through the uncutinized lenticel cells; (4) the acid condition of the moisture on the surface of the fruit. It is believed that the first mechanism is the important one under commercial conditions. The second is unimportant commercially and the importance of the fourth is undetermined. The third undoubtedly occurs, but its presence is unnecessary since the volatile products of the fruit alone will give sufficient stimulus to infection.

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Lenticel infections can occur at cold storage temperatures, and are usually not incipient at the time of storage. Lenticel infections started in cold storage develop rapidly on removal to higher temperatures, and may be the cause of reported instances of sudden decay of lots of fruit when taken from storage on eastern terminals. Infection at wounds was shown to occur in cold storage. The value of cold storage, from the decay standpoint, lies in its retardation of development rather than in the prevention of infection.

Holding the fruit under dry, warm conditions for several days previous to washing may be a practical method of reducing the number of susceptible lenticels, but further investigation is necessary before any definite recommendation can be made. The hastened senescence and increased water loss are the obvious objectionable features of such a treatment. Dye tests would indicate that fruit responds better to such treatment at the time of picking than after being held in cold storage.

SUMMARY

1. Two types of grower-lots in relation to the loss from decay originating from lenticel infections by *Penicillium expansum* were found: (1) those with severe injury every year; (2) those suffering occasional losses, ranging from sporadic severe injury nearly to the hypothetical condition of fruit free from this type of invasion.

2. The methylene blue dye test of Clements and the decayed-tissue test of the writers are potentially valuable indices of the susceptibility of a given lot of fruit to lenticel infections.

3. There are three main factors affecting the incidence of lenticel infections by *P. expansum.* In the decreasing order of their importance these are: (1) susceptibility of the lenticels; (2) number of blue-mold spores on the fruit; (3) conditions influencing the process of infection. Only the first two are thought to be limiting factors under commercial conditions, and the second not generally so.

4. The susceptibility of the lenticels to infection is conditioned by a multiplicity of factors. In these investigations, delayed picking, and storage of fruit at 0° C. previous to washing in heated (43-49° C.) solutions increased their susceptibility for the apple as whole; lenticels in bruises and pressure spots were more susceptible than in uninjured areas. Holding the fruit in the orchard (especially when held dry) previous to storage usually decreased susceptibility of the lenticels to infection; a more intense treatment of the same type with dry heat decreased susceptibility in all cases. A number of other environmental factors appeared to be neutral in effect.

5. The greater the spore load of the fruit surface the greater the chance of lenticel infections.

6. There are four potential stimuli of germination of the spores previous to infection of apples through lenticels: (1) volatile products of the apples; (2) exosmosis of nutrients through the uncutinized lenticel cells; (3) juice from adjacent decayed fruit; (4) the acid condition of the moisture on the surface of the fruit. The first condition is thought to be the important one under commercial conditions.

7. Presumptive evidence is presented that the promotion of lenticel infection by contact with adjacent decayed apples is due to enzymatic action on the uncutinized cells of susceptible lenticels, as well as to stimulus of spore germination.

8. No single factor studied seemed to cause a sufficient increase of lenticel infections to account for the high percentage of such decay frequently found in commercial lots. Probably such fruit has a high contamination of blue-mold spores and also has been exposed to the joint action of several conditions which increase the susceptibility of the lenticels to infection.

9. Complete control of lenticel infections by the reduction of the number of susceptible lenticels through modification of cultural practices is largely impractical, because of the many conditioning factors of the environment and of the fruit itself; reduction of the incidence of such infections by this method may be found practical. Further study may show the efficacy of a brief exposure of the fruit to dry heat previous to packing in order to decrease the number of lenticels susceptible to infection.

10. At present it is advisable to avoid as many conditions known to predispose to lenticel infections as practicable. Avoid delayed picking, bruising, the holding of fruit in the orchard during rainy weather, and the storage of apples at 0° C. previous to washing in heated tanks. On the other hand, leaving the fruit in the orchard in dry weather may decrease the amount of lenticel infection. Reduction of spore load by sanitation in the orchard and packing shed is necessary.

11. The value of cold storage in reduction of the amount of blue-mold decay lies in its retardation of development rather than in prevention of infection. Infections of lenticels and mechanical injuries occur at cold storage temperatures. The rapid development, on removal of fruit to higher temperatures, of incipient lenticel infections unnoticeable in cold storage may be the cause of the sudden increase of decay in certain cases on eastern markets.

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